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Stress fractures result from repetitive loading and have been regarded as a mechanical fatigue-driven process. However, data suggests that increased remodeling precedes the occurrence of bone microdamage and stress fractures, suggesting a central role for increased intracortical remodeling in the pathogenesis of stress fractures. Our ongoing experiments test the hypothesis by pharmacological inhibition of bone remodeling will slow the subsequent accumulation of microdamage, diminishing the severity of the stress fracture. We are using a bisphosphonate (BP) in the rabbit tibial stress fracture model, to test the hypothesis that reactive remodeling within the cortex drives the development of stress fractures. Ongoing studies suggest that BP antiresorptive therapy reduces the intensity of the stress fracture response, as indicated by techneitum bone scans. This effect was most pronounced with short-term loading (3 weeks), with ^{99}technetium uptake in BP treated animals reduced approximately 25 percent from control levels; this effect was diminished by 6 weeks of loading. Reduction of bone ^{99}technetium uptake at 3 weeks in drug-treated animals is consistent with suppression of the acute activation of new intracortical resorption foci by bisphosphonates. The implication of this suppression of the later accumulation of bone microcracks, and the evolution of final stress fracture, are unknown.

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INTRODUCTION

Because the continuing lag in completeing the animal portion of these experiments, we requested and were granted a no cost extension for the current project period, through June, 2002. Thus, we are still in year 3 of the grant. This lag resulted from continuing staffing and equipment problems at the Henry Ford Hospital. In addition, we (Mount Sinai Medical School) recently negotiated a subcontract from the Henry Ford Hospital, to allow us to begin histological processing of the large specimen back-log which accured at the Henry Ford Hospital as a result of personnel loss.

The ensuing progress report is essentially unchanged from the revised report, prepared and submitted to the Department of Defense in February, 2001. Key changes are italicized.

Stress fractures result from repetitive loading and have been regarded as a mechanical fatigue-driven process. However, histopathological data and experimental data from our laboratory suggests that increased remodeling precedes the occurrence of bone microdamage and stress fractures, suggesting a central role for increased intracortical remodeling in the pathogenesis of stress fractures. Thus, we propose that stress fracture occurs through a positive feedback mechanism, in which increased mechanical usage stimulates focal bone turnover, resulting in a local increase in porosity. Microdamage accumulation and stress fractures result from continued cyclic loading of this transiently osteoporotic bone. These experiments test the hypothesis by pharmacologically inhibiting the bone remodeling response; the subsequent accumulation of microdamage and the severity of the stress fracture can be diminished. This hypothesis is being tested experimentally in the rabbit tibial stress fracture model, which was developed in our laboratory. To test the hypothesis that reactive remodeling within the cortex drives the development of stress fractures, the effect of remodeling suppression using a bisphosphonate on the accumulation of bone microdamage and diminishing the severity of stress fracture will be examined. Outcomes of these experiments will be assessed using bone scintigraphy, histomorphometry, and biomechanical approaches.

SUMMARY OF RESEARCH

Our objectives in these experiments are to use the rabbit tibial stress fracture model: (1) to determine at the whole bone level whether bisphosphonate inhibition of intracortical remodeling attenuates the increase in focal bone ^{99m}Technetium uptake which characterizes the development of stress fracture, (2) to determine at the tissue level whether bisphosphonate inhibition of intracortical remodeling decreases the accumulation of cortical bone microdamage which occurs at the site of stress fracture, and (3) to determine how stress fracture compromises mechanical properties of long bones and whether pharmacological inhibition of remodeling can offset that functional deficit.

The project is proceeding toward the goals originally outlined for Year 3, with all procedures continuing. We continue to be behind schedule for initiating the histological analyses of stress fracture tibiae, due to personnel loss. Specifically, our histology technician left the Henry Ford Hospital in December 1999 and we have been unable to recruit a qualified candidate to replace her due to the very tight job market in this area. *We have implemented an alternative plan to begin to remediate the large back log of specimens. In September,2001, we negotiated a subcontract from the Henry Ford Hospital, to allow us to begin histological processing and analyses of specimens in Dr. Schaffler's laboratory, at the Mount Sinai Medical Center in New York. There they will be processed and subjected to histomorphometric analysis, as per our original protocol. Dr Schaffler has recruited a post-doctoral research fellow, who will start in January 2002; this individual's major responsibility will be to analyzes the current experiments.*

Results to date:

The ensuing progress report is essentially unchanged from the revised report, prepared and submitted to the Department of Defense in February, 2001. We have completed experimets on 24 additional animals between February to September 30, 2001. Analyses for the bone scan data for these additional animals ares not yet completed and are not included in our summary presentation of the data.

Bone scan procedure:

We have developed a standardized the procedure for ^{99m} Technetium injection, scanning, and quantification to control for variability between animals and among groups. The animals were each injected with 3 mCurie of ^{99m}Tc starting at 2:00 PM in a predetermined sequence. The isotope was administered IV, with an injection time of about 5-6 minutes per rabbit. Scans were conducted 3 hours later to image the bone phase of ^{99MDP}Technetium.

The rabbits are scanned using a General Electric STARCAM System with a pinhole collimator and the data archived on optical disk for later analysis. Prior to scanning, the rabbits are anesthetized with ketamine -xylazine and the lower extremities are placed into one of two positioning devices. The anterior positioning device captures the lower leg at the distal tibia and holds the legs with the anterior aspect of the leg toward the collimator. The lower limbs are slightly separated, parallel, and level. The positioning for the medial view places the lower extremity of the animal in a device that positions the legs at a 60° angle and level to the collimator. Total time to obtain both A-P and M-L images of each rabbit is about 12-15 minutes.

A standardized area is used to determine a region of interest at the stress fracture site for the anterior view. This region of interest has the same dimensions for all animals and provides an average count per pixel of isotope incorporation within the standard area. The same standardized area is also used to determine a background level of isotope incorporation. This region is distal to the site of stress fracture and also has the same dimensions for all animals. An average count of isotope incorporation per pixel is obtained within the background area. The

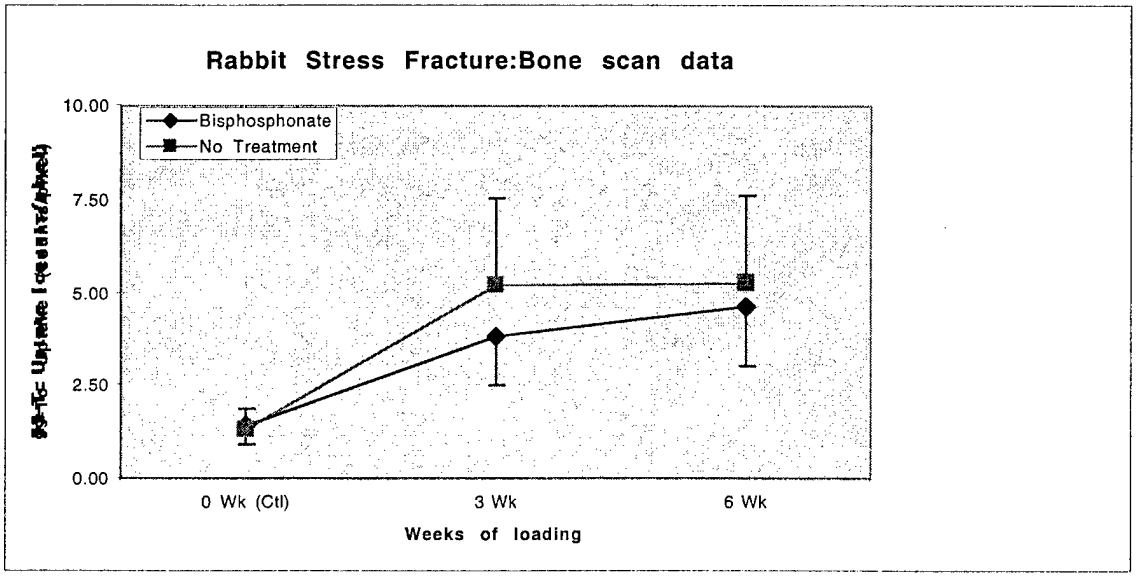
average counts per pixel in the stress fracture region of interest are normalized by dividing by the average counts per pixel in the background region (Average Counts per Pixel Stress Fracture Region of Interest/Average Counts per Pixel Background Region). The mean value for each time period is compared between the bisphosphonate-injected and saline-injected groups.

Results:

The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the stress fracture response, as indicated by techneitum bone scans. This effect was most pronounced with short-term loading. Based on analyses of the experiments to date, bisphosphonate treatment produces a small reduction in technetium uptake relative to vehicle treated controls at all loading time periods (treated vs. control 26 percent lower at 3 weeks of loading ($p<.07$) and 13 percent lower after 6 weeks of loading (p=n.s.), Mann-Whitney U-test). These data are summarized in Figure 1.

Reduction of bone technetium uptake at 3 weeks in drug-treated animals is consistent with a suppression of the activation of new intracortical resorption foci by bisphosphonates. It is unclear at this time why technetium uptake in bisphosphonate-treated animal at 6 weeks increases to control levels. Both an "escape" from remodeling suppression, as well as periosteal reaction resulting from long-term loading, could both account for this finding. Resolution of this of tissue mechanism question awaits completion of the histological studies.

Figure 1



YEAR 3: Goals:

The goals of the second year of the project were to continue to mechanically load rabbit hind limbs (with and without pharmacological inhibition of remodeling) on 32 rabbits (16 – 3 week duration experiments and 16 – 6 week duration experiments) to complete mechanical loading experiments for bone scans and histomorphometry analyses.

- Finish animal loading expriments (N-48 animals)
- Perform 64 ^{99m}Tc bone scans on loaded animals
- Analyze bone scans
- Harvest Tissues from these experiments
- Complete histological processing
- Begin histomorphometric analyses of loaded tibiae.

KEY RESEARCH ACCOMPLISHMENTS: YEAR 3

Key finding: The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the stress fracture response, as indicated by techneitum bone scans. This effect was most pronounced with short-term loading.

As noted previously, we are still in year 3 of the grant, so we have not yet completed our goals for the current year. To date, tasks accomplished specific to the grant Statement of Work are:

- Mechanical loading completed on 24 experimental animals, distributed equally between bisphosphonate and vehicle treated animals
- ^{99m}Tc bone scans and analyses were carried out on experimental and control animals to date
- All bone scans performed are analyzed
- All tissue from loaded animals has been harvested
- Histomorphometric analyses delayed due to loss of technician.

Note: Our progress and accomplishments are revised from those originally projected in our statement of work.

We are significantly behind our anticipated goals for this period. This results primarily from delays in Year 1 caused by a physical plant problem at the Henry Ford animal facility, which resulted in more than 6 months delay in the start of work on this project. These delays were detailed in our Year 1 progress report. Because experimental loading take several weeks to complete and relies on one key piece of equipment, this initial delay will propagate through the project, even as attempt to we catch up.

With the assistance of our Bioresources Department to permit the overlap of animal orders and the careful planning and scheduling of experiments, we were able to bring the progress of the mechanical loading of the rabbit hindlimbs closer into in line with expected

goals. However, we remain behind schedule for the entire project for the reasons described above. To date we now have completed mechanical loading and bone scan studies on 48 experimental animals, and have completed 16 non-loaded controls.

We also note that we have had to deal with turnover in key technical personnel, which has further slowed our progress.

REPORTABLE OUTCOMES

Schaffler, M.B.: Bone fatigue and remodeling in the development of stress fractures. In: D.B. Burr and C. Milgrom, eds., *Musculoskeletal Fatigue and Stress Fracture*, Boca Raton: CRC Press. 2001, pp. 161-182

CONCLUSIONS

None to date. Experiments are ongoing.

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Fatigue and repair in bone

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Cyclic loading of bone, as in all materials, leads to failure incrementally through a process known as fatigue [1,54]. In bone, this incremental failure process corresponds to the accumulation of microstructural level failures or microdamage [4,12–14,19,48,49]. Mechanically, the accumulation of microdamage is correlated to loss of material stiffness, or modulus reduction. Studies from our laboratory [4,48–52], recently corroborated by others [9,42,65], show that bone fatigue can occur at strain magnitudes comparable to those measured on living bones in the physiological loading environment during vigorous activity in animals and humans. At these strain magnitudes, the fatigue life to failure for compact bone is extremely long — in the order of 10^7 load cycles, which corresponds to approximately 5–10 years of use in life [10,13]. However, significant amounts of fatigue damage occur throughout the loading history; damage which must be repaired in order not to lead to fatigue failure of skeletal elements. Thus, fatigue damage has both mechanical and biological consequences.

1. Bone: material and microstructure

Microstructurally, bone is a complex material, comprised principally of the fibrous protein type 1 collagen embedded in a mineral matrix comprised primarily of hydroxyapatite crystals of the size order of 50 nanometers. Bone is most commonly formed in layers, or lamellae, in which collagen fiber orientation in each successive layer appears to be at 90 degrees to the previous layer, making bone into a cross-ply laminate like plywood. Each of the layers is approximately 2–5 micrometers in width. During formation, some of the bone forming cells, or osteoblasts, become entombed in the bone to give rise to the tissue-resident bone cells called osteocytes. Around the shaft of a long bone, the tissue is made of large sheets of lamellar bone organized in concentric rings around the entire bone, like tree rings. Lamellar bone can also be organized into smaller tubes of concentric layers of bone, known as osteons or Haversian systems. A schematic summary of bone microstructure is presented in Fig. 1.

Based on its microstructure, bone can be modeled as

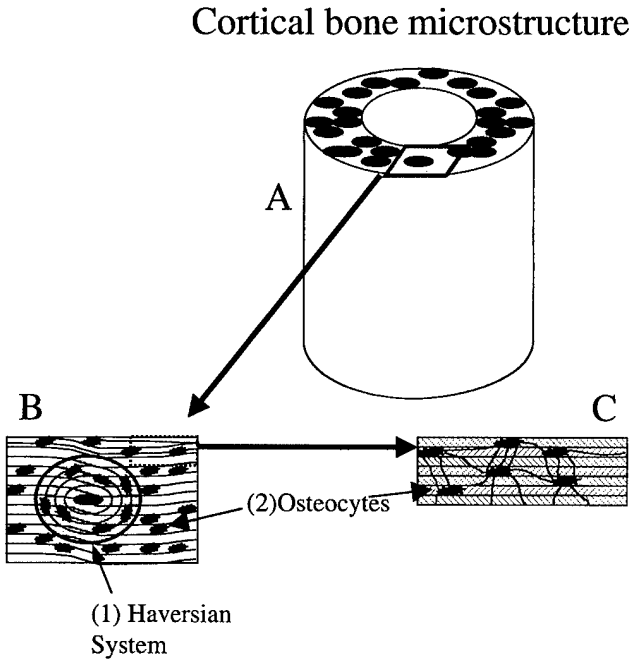

Fig. 1. Schematic representation of bone organization. (A) shows a cylinder from the shaft of a human long bone. Dark gray circles represent the osteons, or Haversian systems; areas of bone between osteons comprise the interstitial bone. (B) is an enlargement of a single osteon (arrow 1) and surrounding region, from within the reference areas shown in (A). The osteon is comprised on concentric lamellae, with a canal at its center (shown in black) which would contain an artery, vein and nerves. Osteons in human bone are approximately 0.2 mm in diameter. Small arrows (2) indicate the stellate shaped osteocytes which reside in the bone matrix. (C) is an enlargement of the reference area shown in (B), indicating the osteocytes within bone and the extensive inter-communication of their cell processes. The bone is shown as layers with alternating orientations for each layer, to represent the cross-ply organization of lamellar bone.

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being a composite material, rather than a monolithic material like aluminum. Accordingly, bone has numerous internal interfaces at which cracks can begin during loading, and at which such cracks might be effectively "isolated" to prevent their coalescence to a fatal crack [12,22,27,48].

2. Fragility and matrix damage in bone

Unlike synthetic engineering materials, bone is capable of detecting and repairing fatigue damage. This repair occurs at the microscopic level, and is distinct from the widely appreciated ability of bone to repair a fracture. Repair of matrix microdamage occurs through a microscopic "drill and fill" process known as bone remodeling (Fig. 2). In this process, bone removing cells, called osteoclasts, tunnel into and remove damaged regions of bone, leaving a tunnel typically about 0.20 millimeters in width and several millimeters in length. Bone forming cells then concentrically fill in the tunnel, forming a completed osteon, with a central canal of about 30–50 micrometers in diameter containing blood vessels and nerves. How remodeling units (tunneling osteoclast followed by osteoblasts) "target" damaged areas of bone is not understood. Osteocytes, the resident cells buried within the mineralized matrix of bone, appear to play a critical role in this process.

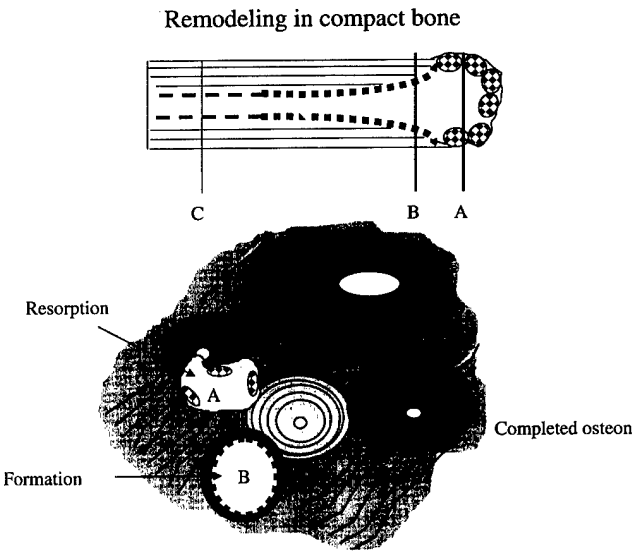

Fig. 2. Schematic representation of remodeling in bone. Upper figure shows a longitudinal section through a remodeling unit in bone. Region A corresponds to the so-called cutting cone at the tunneling end of the remodeling unit, in which large, multinucleated, bone resorbing cells (Osteoclasts, represented as checkered cells) advance through the bone at approximately 40 μm/day. Region B shows the re-filling portion of the forming osteon, lined with smaller, cuboidal-shaped, bone forming cells (Osteoblasts, represented as black squares) which deposit layers of bone. Region C corresponds to the completed region of the osteon, with flattened lining cells lining the central canal. Lower figure shows a diagrammatic cross-section through a remodeling region of compact bone. (A) cuts across the cutting cone of an remodeling system. (B) cuts across a re-filling osteon. (C) shows a cross-section of recently formed osteon.

Left undetected and unrepaired, the accumulation of microdamage in bone leads to compromised mechanical properties and bone fragility. Stress fractures in soldiers, ballet dancers, joggers and other individuals who have increased their levels of repetitive-type physical activities, is widely believed to exemplify damage accumulation exceeding the ability of bone to repair [10,39]. Microdamage accumulation due to a high cycle, low stress type failure occurs in the increased bone fragility associated with aging and osteoporosis; in that instance repeated normal use combined with diminished tissue repair lead to the accumulation of matrix damage and the weakening of bone [39,40,51].

Bone microdamage and fragility are also implicated in bone implant failure [25,26] and fractures associated with long-term usage of drugs that suppress bone remodeling physiology [18,41]. Suppressing remodeling may have deleterious consequences for damage accumulation. Recently, with the introduction to wide clinical usage of drugs which turn off bone remodeling in the skeleton, there have been serious concerns raised about whether pharmacological inhibition of bone remodeling will predispose bone tissue to the accumulation of unremodeled microdamage, leading to increased bone fragility in the population. Thus paradoxically, drugs which solve the problem of bone loss in aging and menopause by reducing bone remodeling may also lead to damage accumulation and tissue fragility as an untoward by-product of their mechanism of action. Accordingly, examination of the factors which influence microdamage accumulation in the skeleton, and those factors which influence its detection and repair are fundamental to understanding skeletal health and disease.

3. How does bone fatigue within the normal range of physiological stresses, strains and cycles?

Bone fractures with relatively few loading cycles when cyclic stresses or strains are large. Carter and coworkers [13,14] showed that bone can fail in fatigue in as few as 1000 to 100,000 loading cycles at strain ranges of 5000–10,000 microstrain. In vivo strain studies, however, indicate that habitual peak physiological strain ranges in living animals are considerably lower, typically less than 1500 microstrain in tension and 2500 microstrain in compression [46,48]. Nunamaker et al. [38] report that high strains, in excess of 5000 microstrain, occur in growing thoroughbred horses when they become muscularly fatigued after racetrack training. Rubin et al. [47], however, have not observed comparably high strains in horses after muscular fatigue. Recently, Burr et al. [11] applied strain gages to the tib-

ial shaft in Israeli soldiers during intensive training regimes, and found that repetitive strains did not exceed the 2500 microstrain level for any voluntary activity, no matter how extreme the regimen. They also observed that after extreme muscular fatigue. strain magnitudes did not change, but strain rates increased significantly.

At physiological strains, in the range of 1500 to 2500 microstrain, the predicted fatigue life to failure of compact bone is extremely long — on the order of up to 10 million load cycles [13]. However, studies by Schaffler and co-workers in our laboratory [48,49] showed that cyclic loading of compact bone at physiological strains associated with vigorous physical activities for more than ten million cycles does not cause failure of compact bone. Nevertheless, in all instances a significant amount of fatigue occurred, evidenced by up to 10 percent stiffness loss in test specimens. Pattin et al. [42] and Gibson et al. [24] have recently reported similar observations. Our studies found that most of the fatigue process occurred early in the loading history, with most of the modulus degradation occurring within the first 1–2 million cycles of loading. Stiffness loss then stabilized for the duration of the experimental loading period and did not progress to failure.

An analogous temporal pattern of fatigue behavior occurs in many fiber reinforced composite materials [1,45]. Under low stress or strain cyclic loading conditions, stiffness loss occurs early in the loading history, corresponding structurally to the initiation of new cracks and voids in the material. Stiffness loss then slows until very late in the loading history, when it again resumes and progresses rapidly to failure. This tri-phasic failure behavior for low stress/strain cyclic loading failure of composite materials, and apparently compact bone as well, stands in contradistinction to the earlier idea that compact bone can be characterized as a material that has linear, progressive loss of stiffness leading to failure [12–14]. Thus, at the low stress/strain levels at which bone is habitually loaded, bone sustains fatigue damage quickly, but that damage does not readily progress to failure.

Finally, it is noteworthy that fatigue fracture did not result at the stresses/strain characteristic of habitual loading, raising questions about the cause of so-called stress fracture. It may be that bone experiences significant periods of higher strain loading under some vigorous activities, leading to more pronounced and progressive fatigue. However, existing in vivo strain gage studies in adult humans and animals argue against this hypothesis.

4. Microdamage in compact bone?

There is a general consensus that fatigue in bone will produce microcracks. With fatigue at relatively modest stresses and strains, bone shows significant modulus degradation. In composite materials, modulus degradation corresponds to matrix damage formation. However, given that bone is a relatively brittle, inhomogeneous material, definitive visualization of matrix damage, and validation that matrix cracking was not a result of microscopic preparation techniques, has been problematic.

Frost [20] reported the first observations of microdamage in bone in human rib samples obtained at autopsy. He described small cracks, with a "linear" morphology, typically on the order of 30–100 μm in length, and also postulated that these cracks could result from fatigue in vivo. Frost's approach for studying microscopic damage in bone still forms the core of fatigue and matrix damage research in bone some 40 years after its original description [7,21,51,52]. His simple and elegant approach was to take a large sample of bone tissue and then bulk stain the entire tissue block in a dye (basic fuchsin) which binds non-specifically to open bone surfaces. After staining, bone would then be sectioned and polished for transmitted light microscopy. Microcracks existing in the bone prior to sectioning would be stained; new cracks introduced during sectioning for microscopic observation would remain unstained and could therefore be readily distinguished as artifact. Recently, the bulk staining approach has been updated to include fluorescent and heavy-metal dyes, allowing studies using confocal microscopy and back-scattered electron microscopy [31,50,63–65].

Microcracks, of the typical linear morphology first described by Frost, have been produced experimentally by applying physiological stresses or strains cyclically to devitalized bone samples [48,49] and in vivo as well [2,8,36,57]. There is little consensus as to what sorts of loading conditions lead to the formation of microdamage in vivo. Microdamage formation with cyclic loading at lower strains typical of normal locomotor loading has been confirmed in the studies previously described. However, we and others have shown that in some loading modes (tension samples, whole bone bending studies), most of the typical linear microcracks occurred late in the fatigue loading history of bones, after more than 30 percent modulus loss [9,52]. Thus, it is clear that there are other levels of matrix failure in bone, which occur early in the fatigue process, and strongly influence its fatigue behavior.

5. What other types of damage occur in compact bone?

While the formation of microcracks will lead to a decrease in bone modulus, numerous experimental studies fail to find a strong correlation between microcrack content (crack number per tissue volume) and the amount of modulus degradation. Number of cracks itself

may be an incomplete descriptor of damage content, since such cracks have dimensions (longitudinal as well as width extents) which are not factored into simple number density measures. Similarly, crack orientation and position relative to microstructure and load may also exert strong effects on modulus degradation. However, there may also be other levels of bone matrix microdamage, beyond microcracks, which result from fatigue loading. Inherently, bone is an hierarchical, inhomogeneous material, and cracks can potentially form at any level in this hierarchical structure. An understanding of the other levels of matrix failure in bone has only recently begun to emerge, facilitated by the development of advanced microscopy techniques.

In recent experiments from our laboratory, human compact bone samples were fatigued in tension to increasing amounts of damage, as evidenced by modulus degradation. Typical linear-type microcracks were rarely observed in the specimens at lower fatigue levels (15% modulus degradation); they were observed routinely at higher levels of fatigue (30% modulus degradation, Fig. 3A). In fatigue-loaded specimens, patches of diffuse basic fuchsin staining of the bone matrix were observed, indicating a fatigue-induced change in bone matrix permeability to the stain. The amount of this diffuse staining increased in direct relation to increasing specimen fatigue levels (Fig. 3B). In their studies of whole bone fatigue in canine long bones, Burr et al., also reported that typical linear microcracks were not observed until more than 30–40% stiffness loss [9]. In summary, these data show that matrix damage processes which are reflected by changes in permeability to basic fuchsin: 1) occur early in the fatigue process, and 2) constitute a better predictor (than typical microcracking) of fatigue and material stiffness loss.

6. What is the nature of bone microdamage within the patches of basic fuchsin staining observed in fatigue loaded bone?

In order to begin to answer questions about the nature of bone microdamage within the patches of basic fuchsin staining seen in fatigued bone, we developed new methods for high resolution study of matrix-level failures in compact bone [4]. Basic fuchsin fluoresces under long wavelength illumination (568 nm excitation), and was found to give excellent high resolution confocal microscopy images of microdamage in bone, allowing examination of foci of fuchsin staining in the same fatigue specimens described above.

Using confocal microscopy we found that the patches of diffuse basic fuchsin staining in fatigued bone were comprised of very fine matrix cracking at the sub-lamellar-level (<5 micrometers) size order in bone. The majority of this damage was seen as patches of interconnected fine cracks, or microscopically comminuted areas (Fig. 4). We also observed occasional foci of dye uptake within regions of identifiable matrix microcracking, for which no cracks could be resolved using confocal microscopy. As the maximum lateral resolution of confocal microscopy is ~200 nanometers, these patches indicate that some damage occurs at even finer levels of bone matrix structure. In the current studies, this diffuse matrix microcracking was the only damage type present at low levels of tensile fatigue in bone. Zioupos and Currey [65], in their studies of fracture toughening mechanisms recently reported similar early mechanisms of matrix failure. In our analyses, we found that the preponderance of this fatigue damage appears as very fine microcracks, or microcomminuted areas. The principal bone matrix structures at the level of organization of

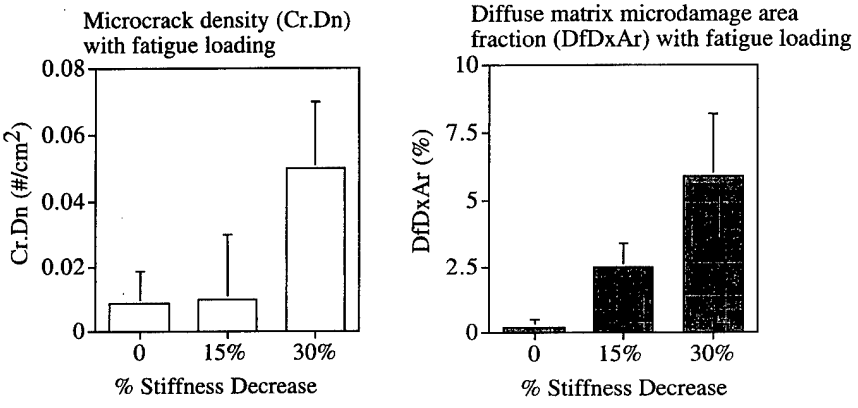

Fig. 3. Microdamage content of human bone tensile fatigue specimens, loaded to two different levels of modulus degradation. (A) Left graph shows number density of microcracks unchanged from control tissue after loading to a 15% decrease in specimen modulus. Crack number increased dramatically after loading to a 30% decrease in specimen modulus. (B) Right graph shows area occupied by diffuse stain area (expressed as a percentage of total tissue area) after fatigue loading to the same levels of modulus degradation. Diffuse damage fraction increases in concert with stiffness loss.

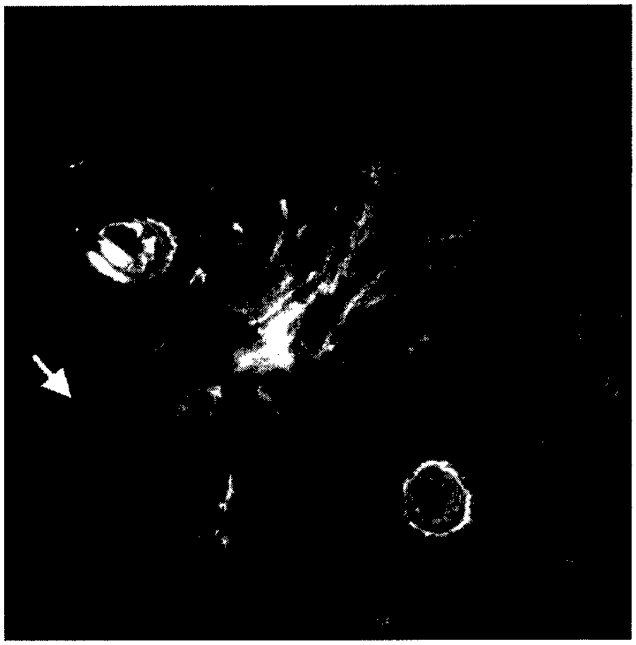

Fig. 4. Confocal photomicrograph of basic fuchsin-stained section of human bone sample fatigued loaded to 30% decrease in specimen modulus (see Fig. 3). Arrow shows a linear microcrack. The bright-staining region near center of the field is a diffuse staining patch (see text for explanation), comprised of large numbers of very small, interconnected cracks. (Photomicrograph field width=560 μm).

these very small cracks in bone are aggregates of hydroxyapatite crystals, known as spherulites, suggesting that early matrix failure in bone might occur principally at the level of these structures [44,58,59].

7. Are similar diffuse matrix microdamage processes seen in human bone?

Given that the previous experiments indicate that diffuse matrix microdamage processes are a major mode of early fatigue damage, a series of normal (not fatigue-loaded) specimens of human bone were examined to determine whether this type of damage would be seen in vivo. We recently reported a strong, exponential age-related increase in the amount of microdamage (defined as typical linear microcracks) present in human femoral diaphyses, beginning from about age 40 [51]. Damage accumulation was also observed for the femoral neck cortex; microcracks are also seen in the human spine [61]. These studies suggest that there is a chronological point at which microcracks accumulate more rapidly than intrinsic processes can repair them. In compact bone, the extent to which microdamage accumulation with aging represents either a change in tissue susceptibility to damage or an alteration in the ability of the tissue to perceive and/or react to microcracks is currently unknown.

Basic fuchsin stained sections from these same specimens were examined using the confocal microscopy approach to assess diffuse matrix microdamage content. Foci of diffuse damage were observed most often in subjects greater than 50 years of age, consistent with the age at which our previous study suggests that remodeling–repair mechanisms become ineffective in detecting or keeping pace with the accumulation of damage in bone. By analogy to composite materials, the accumulation of microdamage in the cortices of long bones would be expected to significantly weaken the bone, and in particular, reduce its resistance to fracture. Certainly, it is well known that older bone is weaker and less tough than younger bone. The role of microdamage accumulation in this age-related loss of strength and toughness is likely to be significant.

Together, these data indicate that diffuse bone matrix microdamage is a major mechanism of damage in compact bone at low levels of fatigue. It also appears in association with in vivo loading. As this damage phenomenon in bone has only recently been recognized, its physiological significance is currently not completely understood. However, it is clear that it may be an important stimulus to certain forms of matrix turnover and repair.

8. How does stress type influence fatigue damage mode in bone?

The relative influence of different strain modes on damage and strength in compact bone was until recently poorly understood. Boyce et al. [4] took advantage of the non-uniform strain field produced by four-point bending to examine this question. They conducted four-point bending fatigue tests of human compact bone samples by cyclically loading to a single level of stiffness degradation, and then measured bone microdamage type in each of the principal stress regions (tension, compression and along the neutral axis). They found that in regions subjected to tensile strains, the bone showed predominantly focal regions of diffusely increased basic fuchsin staining (i.e. diffuse microdamage), consistent with the mineral-collagen disaggregation discussed above. In compressive strain regions, the tissue developed linear microcracks in interstitial areas similar to those originally described by Frost in 1960. While the mechanism for this cracking remains unknown, internal buckling, or kink-bands, seems a likely possibility. Fine, tearing-type ("wispy-appearing") cracks were observed near and in the plane of the neutral axis, which were not associated with interfaces inherent to bone microstructure. Other minor damage morphologies (sector stained osteons, delamination of regions of lamellae, intraosteonal cracking) were observed, but their distribution was unrelated to local strain field. Thus, in fatigue of human compact bone, the principal mechanisms of

matrix failure (i.e. linear microcracks, diffuse damage foci and tearing-type microdamage) is strongly dependent on local strain type mode.

9. Does remodeling repair microdamage in compact bone?

Repair potential is inherent to all biological systems. Numerous investigators have suggested that a primary function of osteonal remodeling is reparative: remodeling serves to remove and replace fatigue damaged regions of compact bone [8,20,21,31,32,34,49,51]. Corollary hypotheses regarding the mechanisms by which microdamage may signal or direct a local remodeling response have also been discussed widely in the literature. These ideas include micro-streaming potentials generated in association with crack propagation [33] and microcrack damage to osteocyte canalicular processes [2,12,21]. Despite the widely held nature of the concept that bone remodeling functions in the repair of microdamage, empirical data demonstrating this basic physiological mechanism are scant.

Burr et al. [8] and Mori and Burr [36] showed experimentally that bone resorption spaces are associated with remodeling of linear microcracks from canine compact bone. Recently, Bentolila et al. [2] in our laboratory, adapted the ulnar bending system developed by Torrance et al. [56], for use as an in vivo fatigue system in adult rats. In this system, axial loads are applied to the carpus and elbow of adult Sprague–Dawley rats; bending moments are induced because of the inherent curvature of the ulna. Experiments are performed under load control. Fatigue levels are monitored as changes in whole bone stiffness. Bentolila et al. [2] found that bone remodeling was associated with both linear microcracks and with areas of diffuse matrix damage, and was effective in removing both damage types from the bone.

10. How might matrix damage be detected in bone?

One of the central mysteries in the process of bone remodeling is the mechanisms by which groups of osteoclasts target regions of bone for resorption. Activation of resorption foci is increased as a result of disuse, matrix microdamage caused by fatigue, and recent devitalization of bone. In the cases of remodeling of recently devitalized bone and repairing matrix injury, there is an obvious need for osteoclastic cutting cones to accurately target their resorptive activities to sites of injury. The stimulus to activate and then target osteoclasts is unknown.

It is quite reasonable to presume that osteocytes, the only cells embedded in the bone matrix, would be affected by processes that damage the bone matrix. These cells are highly responsive to mechanical loading [30,53]. In several instances in which osteocytes are absent from bone, fatigue failures will occur; examples include radiation-induced death of osteocytes [35], allograft bone [3] and avascular necrosis [28]. Dunstan et al. [17] showed that the absence of osteocytes is associated with hip fracture. Osteocytes are focally lost in areas of microcrack accumulation in aging human bone [43].

Osteocytes are widely and extensively distributed throughout the bone matrix. Their elongated cell processes infiltrate throughout the matrix of bone through small, ~0.1 μm wide tubes known as canaliculi. Osteocytes are attached to their surrounding bone matrix and to their neighboring cells through electrical connections known as gap junctions [15,16]. Matrix disruption from microdamage can be expected to directly injure osteocytes, disrupt their attachments to bone matrix, interrupt their communication through canalicular cellular and fluid flow processes or alter their metabolic exchange.

The involvement of osteocytes in bone fatigue and remodeling was recently examined by Verborgt et al. [60], using the rat in vivo fatigue model described above. They found that with fatigue in vivo, osteocytes surrounding microcracks are injured and begin an ordered cell disintegration process following a genetically regulated program. This cell suicide process, or regulated cell death, is known as apoptosis. Regulated cell death is the ubiquitous biological process by which cells that have reached the end of their functional life spans break down [6,23,29,62]. The cell breakdown products are key signaling molecules targeted by phagocytic (debris eating) cells throughout the body [62]. The cells that resorb bone, the osteoclasts, belong to the phagocytic cell family. Osteocyte apoptosis has been observed in other metabolic situations associated with bone resorption [5,37,55]. Thus, osteocytes, and in particular the events surrounding their death, appear to provide a key part of the signaling process by which osteoclasts target microdamaged bone for removal and focal repair.

In summary, under cyclic loading bone behaves like a composite material, with fatigue loading giving rise to matrix-level microdamage and concomitant degradation of material properties. Fatigue and resulting microdamage occur readily even at the modest stresses or strains to which bone is subjected during its habitual physiological use. However, at low stress/strain levels, microdamage does not readily progress to failure. Unlike synthetic engineering materials, bone is capable of microscopic repair at the level of matrix microdamage, through a coordinated multi-cellular process (i.e., bone remodeling) which can remove and replace damage foci before they coalesce and lead to catastrophic failure (i.e., fracture) of a whole bone. Recent data suggest that the osteocytes play a pivotal role in the detection of matrix microdamage in bone, and signal its removal, through a

process of cellular injury and resulting regulated cell death.

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CHAPTER 11

Bone Fatigue and Remodeling in the Development of Stress Fractures

Mitchell B. Schaffler

CONTENTS

INTRODUCTION

Stress fractures result from repetitive loading and occur commonly among physically active individuals. Stress fractures are not associated with a specific history of trauma. Rather, they are frequently reported in soldiers, ballet dancers, joggers, and other individuals who have increased their levels of repetitive-type physical activities.[4,28,30,52,55,74-76,78,81] As such, they have been often regarded as a mechanical fatigue-driven process. Stress fractures are ranked between the second and eighth most common running injury, with incidences reported between 4 and 14%.[48,59] Rates of occurrence of stress fracture in the U.S. military were reported by Jones et al.[59] to be in the range of less than 4%. However, recent studies by Hise et al.[52] found that stress

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fracture incidence among female soldiers in basic training was considerably higher, at nearly 8%. In other military training environments, such as the Israeli army, the incidence of stress fracture among soldiers has been reported as high as 31 percent.[74,75]

Clinically, stress fractures present as bone tenderness, often with radiographic evidence of a periosteal callus; less frequently observed is occurrence of an actual fracture line.[30,81,109] Typically, stress fractures occur after four to six weeks of increased activity. This is estimated to correspond to about 100,000 load use cycles.[24,30] In recent years, diagnosis of stress fracture has shifted from radiology to bone scintigraphy using ^{99m}technetium (^{99m}Tc).[46,74-76,91,109,114,115]

There are two hypotheses regarding the cause of stress fractures. One hypothesis holds that stress fractures are the result of development, accumulation, and growth of microcracks within the bone.[20-25,29,35,80] In this view, stress fractures are considered a purely mechanical damage occurrence, i.e., fatigue failure of the skeleton. An alternative hypothesis models stress fracture as a positive feedback mechanism: increased mechanical usage stimulates bone turnover, which results in focally increased bone remodeling space (porosity) and decreased bone mass. With continued loading of this focally, transiently osteopenic bone, local stresses are markedly elevated, leading to accelerated damage and failure. Fracture is the result of continued repetitive loading superimposed on the decreased bone mass caused by more and larger resorption spaces.[30,58,68,97,98]

DOES BONE FATIGUE WITHIN THE NORMAL RANGE OF PHYSIOLOGICAL STRAINS AND CYCLES?

Bone can fracture with relatively few loading cycles when cyclic stresses or strains are large. Carter and Caler[20,21] showed that bone can fail in fatigue in as few as 1000 to 100,000 loading cycles at strain ranges of 5000 to 10,000 microstrain (0.5 to 1 percent deformation). However, *in vivo* bone strain studies indicate that habitual peak physiological strain ranges in living animals are considerably lower, typically less than 1500 microstrain in tension and 2500 microstrain in compression.[62,92,93] Very high bone strains (in the range of 4000 to 5000 microstrain) in muscularly fatigued, growing racehorses have been reported by Nunamaker et al.[80] However, other studies have not observed comparably high strain levels in race horses.[93,94] Recently, Burr and co-workers[18,44,53] applied strain gages to the tibial shafts in Israeli soldiers during intensive training regimes and found that repetitive strains did not exceed 2000 microstrain for any voluntary activity, no matter how extreme the regimen. They also observed that after extreme muscular fatigue, strain magnitudes did not change but strain rates increased significantly.[44] In summary, these data indicate that maximum bone strains *in vivo* during vigorous activities, in humans and in animals, are in the range of about 2000-2500 microstrain.

HOW DOES BONE BEHAVE WHEN FATIGUE-LOADED AT LOWER, MORE PHYSIOLOGICAL STRAINS?

At physiological strains in the range of 1500 to 2500 microstrain, the *predicted fatigue life to failure* of compact bone (defined as fracture) is extremely long — up

to 10 million load cycles. However, Schaffler et al.[97,98] showed that during cyclic loading at such low strains as encountered in habitual loading, bone sustains a significant amount of fatigue damage. This fatigue is evidenced by up to 10% stiffness (modulus) loss in bone test specimens over the first few hundred thousand cycles of loading. A number of other studies have since reported similar observations for fatigue in bovine, canine, equine, and human bone.[17,47,85,100] The mechanical loss of material stiffness, or modulus reduction, during fatigue is correlated to the accumulation of microdamage. All of these studies found that the fatigue process begins early in the loading history, with most of the modulus degradation occurring within several hundred thousand cycles of loading. Stiffness loss then stabilizes for the duration of the experimental loading period and does not progress to failure for up to several million load cycles (Figure 1). Thus, at the levels of stress and strain which are habitually developed *in vivo*, the fatigue life to failure for compact bone is extremely long — 1 to 10 million load cycles, which corresponds to approximately five to ten years of use in life. However, significant amounts of fatigue damage occur throughout the loading history. This damage must be repaired in order to avoid failure of skeletal elements. It should also be noted that strain rate, or the rate at

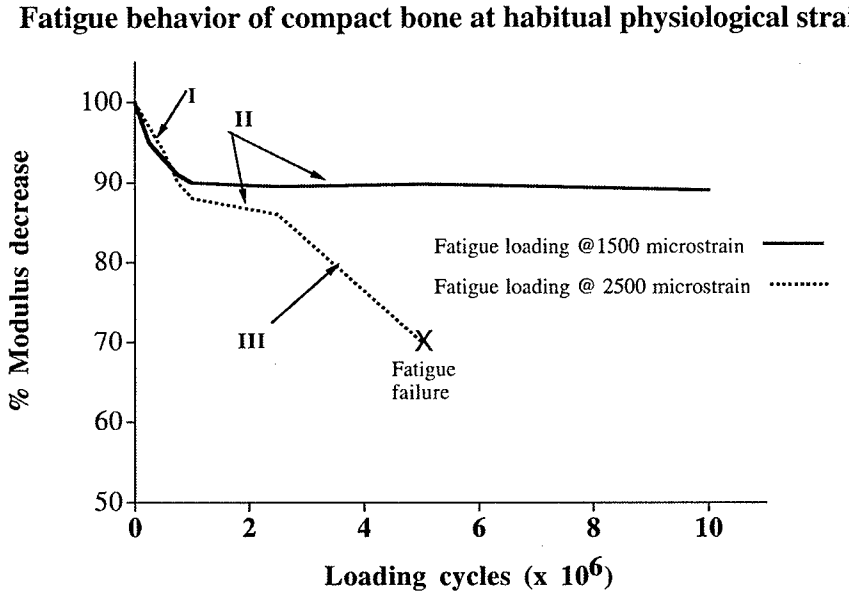


Figure 1 Summary of fatigue behavior of compact bone loaded at two strain levels characteristic of the physiologic loading environment. At the lower strain, characteristic of rapid walking, bone sustains damage and loses stiffness (shown as percentage decrease from the initial elastic modulus) early in its loading history (phase I). Stiffness loss then slows and remains stable (phase II) for up to 10 million cycles. At 2500 microstrain, the strain level characteristic of normal running, bone shows a similar early degradation of modulus (I). Damage accumulation then slows and remains stable for 1 to 2 million cycles (II). At this higher strain, however, modulus degradation will resume and progress to fatigue failure (phase III) after several million cycles of loading.

which peak strains are generated in bone, has a significant effect on damage accumulation. In laboratory fatigue tests, loading at strain rates characteristic of running were more damaging to bone than loading at lower rates, regardless of the magnitude of strain or load.[97]

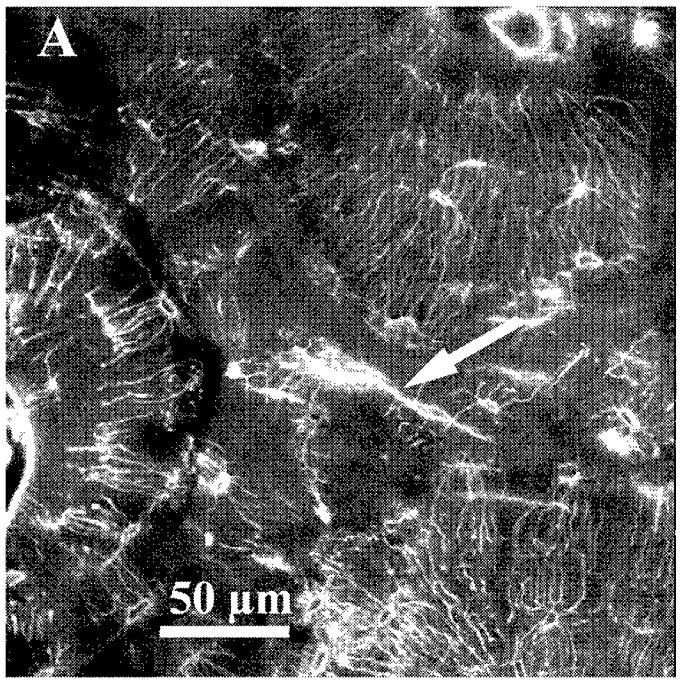
The key point of these data is that bone readily sustains fatigue damage at modest stresses or strains. An analogous temporal pattern of fatigue behavior occurs in many fiber–reinforced composite materials.[1,89] Under low stress or strain cyclic loading conditions, stiffness loss occurs early in the loading history, corresponding structurally to the initiation of new cracks and voids in the material. Stiffness loss then slows until very late in the loading history, when it again resumes and progresses rapidly to failure. This three-phase failure behavior for low stress/strain cyclic loading failure of composite materials, and apparently compact bone as well, stands in contradistinction to the earlier idea that compact bone can be characterized as a material that has a linear, progressive loss of stiffness leading to failure. Thus, at the low stress/strain levels at which bone is habitually loaded, bone sustains fatigue damage quickly, but that damage does not readily progress to failure.

FATIGUE MICRODAMAGE IN COMPACT BONE

Loss of stiffness with fatigue loading is direct mechanical evidence for the existence of damage within the matrix in composite material such as bone.[1,8,22,85,97,98,100] However, given that bone is a comparatively brittle, inhomogeneous material, it has been problematic to visualize matrix damage and validate that matrix cracking is not an artifact of microscopic preparation techniques.

Frost[40] reported the first observations of microdamage (small, 30 to 100 μm-long cracks) in human rib samples obtained at autopsy. He suggested that such microcracks result from fatigue *in vivo*. Frost's simple and elegant approach for visualizing microscopic damage in bone is still central to bone fatigue and matrix damage research some 40 years after its original description.[11,17,47,101,104] Large blocks of bone tissue were stained in a dye (basic fuchsin) which binds non-specifically to open bone surfaces prior to histological sectioning. Microcracks existing in the bone prior to sectioning were stained; new cracks introduced during sectioning for microscopic observation remained unstained and could therefore be readily distinguished as artifact. This bulk staining approach has been updated to include fluorescent and heavy metal dyes, allowing studies using confocal microscopy and electron microscopy.[64,99,103]

Bone microcracks, of the typical linear morphology first described by Frost (Figure 2), have been produced experimentally by applying physiological levels of stress or strain cyclically to devitalized bone samples[17,19,97,98,100] and *in vivo* as well.[5,15,77,108] Moreover, bone is a hierarchical, inhomogeneous material, and cracks can potentially form at any level in its microstructural organization. Thus, it is clear that there can be other levels of matrix failure in bone which occur early in the fatigue process and strongly influence its fatigue behavior. In experiments from our laboratory,[100] human compact bone samples were fatigued to increasing amounts of damage, as evidenced by modulus degradation. Typical linear-type microcracks (Figure 3a) were observed rarely in specimens at lower fatigue level (15% modulus



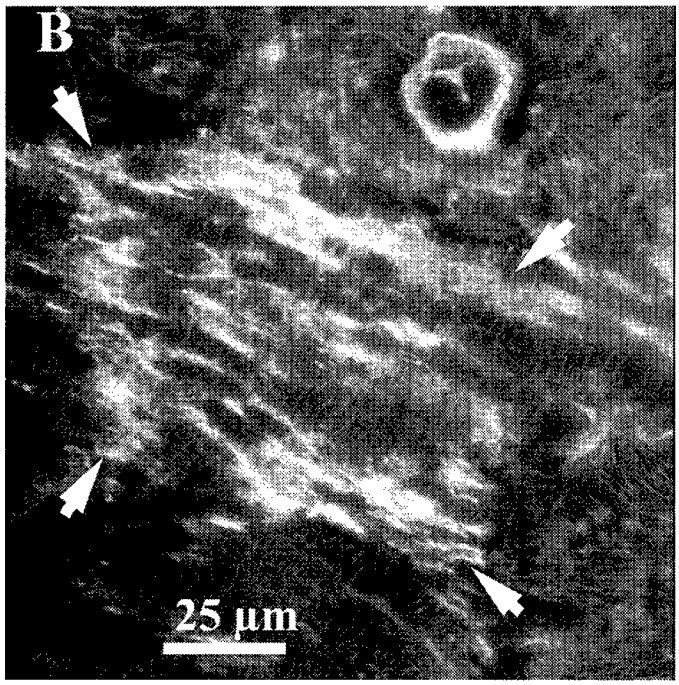


Figure 2 Confocal photomicrographs of microdamage in human bone samples. Upper panel (A) shows a linear microcrack (arrow) typical of that first described by Frost.[40] Lower panel (B) shows a higher magnification view of a region of diffuse matrix damage, comprised of large numbers of very small microcracks.

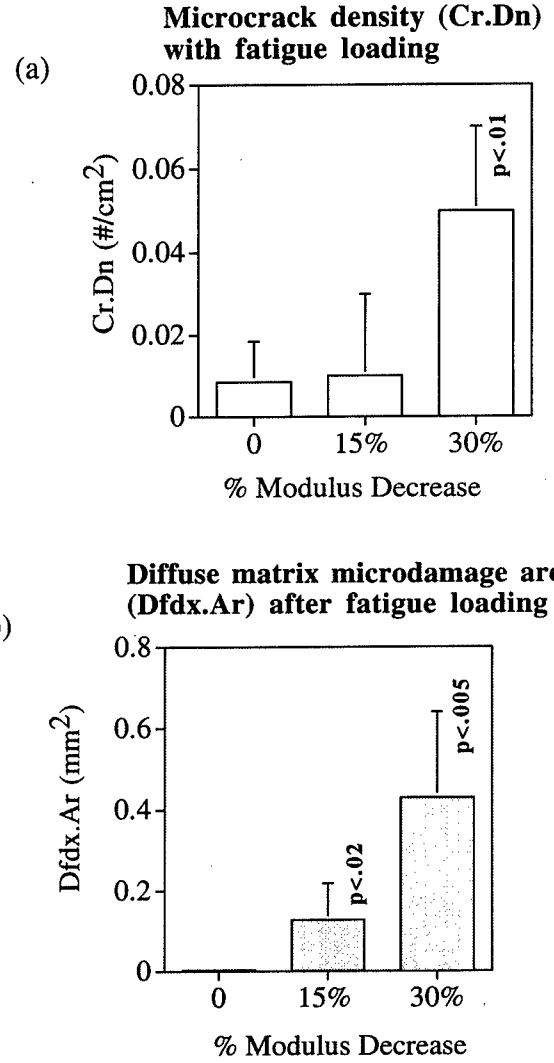


Figure 3 Linear microcrack density (Cr.Dn) and diffuse damage content (Dfdx.Ar) in human bone specimens experimentally loaded to increasing levels of fatigue. a. For linear microcracks, increased Cr.Dn occurs after a 30% modulus decrease. b. In contrast, diffuse damage content increases in direct relation to increasing amounts of fatigue in these samples.

loss) but were observed routinely at higher levels of fatigue (30% modulus degradation). In studies of whole bone fatigue in canine long bones, Burr et al.[9] also reported that linear microcracks were not observed until 15% stiffness loss. However, in fatigue-loaded human bone specimens, patches of diffuse basic fuchsin staining of the bone matrix were observed at all fatigue levels, indicating a fatigue-induced change in bone matrix permeability to the stain. The amount of this diffuse staining increased in direct relation to increasing specimen fatigue levels (Figure 3b).

Confocal microscopy showed these patches of diffuse basic fuchsin staining in fatigued bone to be comprised of very fine matrix cracking at the sub-lamellar level (<5 μm) size order in bone. (Figure 2). Occasional foci of dye uptake were observed within regions of identifiable matrix microcracking, for which no cracks could be resolved using confocal microscopy. As the maximum lateral resolution of confocal microscopy is ~200 nanometers, these foci indicate that some damage occurs at even finer levels of bone matrix structure. Zioupos and Currey,[113] in their recent studies of fracture toughening mechanisms in bone, reported similar early mechanisms of matrix failure. The principal bone matrix structures at the level of organization of these very small cracks in bone are hydroxyapatite crystals and their aggregates, suggesting that early matrix failure in bone might occur principally at the level of these structures.

In summary, compact bone undergoes fatigue and sustains matrix-level damage as a result of cyclic loading at the magnitudes of stress or strain that can be generated with habitual physiological activities. However, at these same stresses/strains, fatigue does not progress to failure within a time frame consistent with the development of stress fractures *in vivo*. These data suggest that other mechanisms must be involved in the development of so-called fatigue or stress fractures *in vivo*.

Studies show that different amounts of fatigue in compact bone lead to different amounts of microdamage, but also to different qualities of the damage present (i.e., diffuse matrix microdamage early in fatigue; typical microcracking later in fatigue). It is well established in materials science that microdamage content (quality and quantity) compromises the residual (remaining) mechanical properties of a material. Diminished residual properties in bone after fatigue were first demonstrated by Carter and Hayes.[22] In order to assess how different amounts and types of damage with different levels of bone fatigue alter functional-mechanical properties, Boyce et al.[8] examined the residual properties of human compact bone after fatigue, using matched contralateral femurs to those used in fatigue experiments described in the preceding section. After completion of fatigue loading, specimens were tested monotonically to failure. Residual properties of ultimate stress (strength), ultimate strain, and work to fracture were measured from stress-strain curves. Among specimens loaded to the lower level of fatigue (15% modulus decrease), residual stress, strain, and work to fracture were reduced in general proportion to the amount of modulus degradation. In contrast, bone specimens fatigued to greater levels (30% modulus decrease) showed losses of ultimate strength and work to fracture far greater than expected based on the stiffness changes in these specimens (67 and 76% reductions, respectively). Most striking, however, is that bone specimens fatigued to the higher level of fatigue showed effectively no post-yield deformation (Figure 4). In other words, the accumulation of fatigue damage caused a disproportionate loss of the ability of bone to withstand a catastrophic fracture.

REMODELING AND REPAIR OF MICRODAMAGE IN BONE

Unlike synthetic engineering materials, bone is capable of detecting and repairing fatigue damage at the microscopic level. Numerous investigators have suggested that

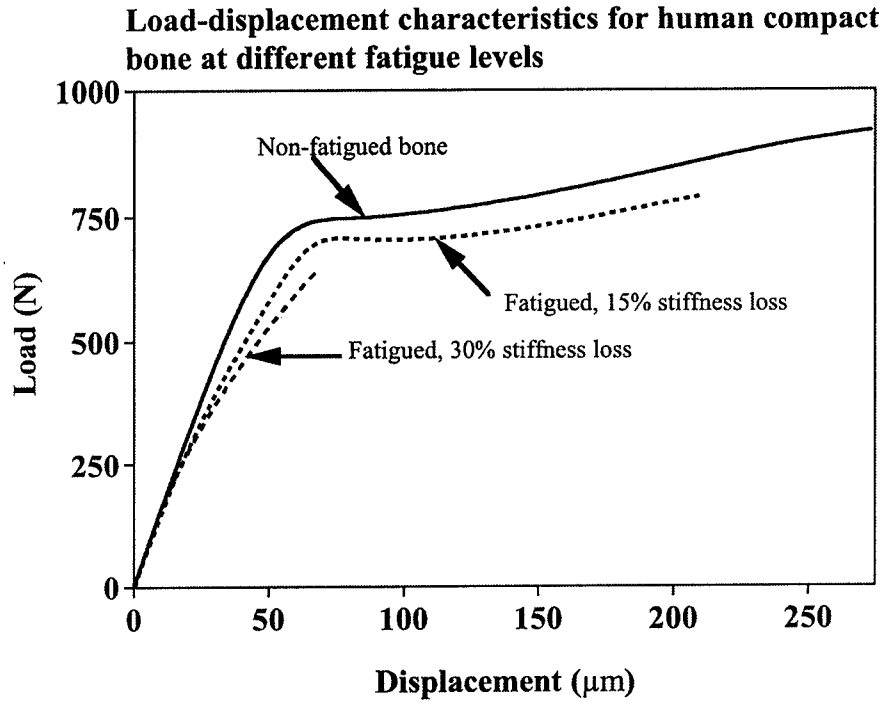


Figure 4 Residual mechanical properties for bone specimens after different amounts of fatigue. Non-fatigued bone shows well-defined elastic, yield, and plastic regions of the loading curve. At a modest level of fatigue (15% modulus decrease), the bone mechanical properties are reduced in close proportion to the induced fatigue level. At the higher fatigue level (30% modulus loss), bone stiffness and strength are reduced proportionally. However, the load-displacement curve no longer shows a yield point or any post-yield region. These data show that higher levels of fatigue cause a disproportionate loss of bone's fracture toughness, or the ability of bone to withstand fracture.

a primary function of osteonal remodeling in the adult skeleton is reparative: remodeling serves to remove and replace fatigue-damaged regions of compact bone.[5,15,19,40-42,68,69,77,84,97,98,101] Specifically, repair of matrix microdamage occurs through a microscopic "drill and fill" process, in which osteoclasts tunnel into bone and remove damaged regions. Osteoblasts then concentrically fill in the resorption space, forming a completed osteon. The remodeling repair response is summarized schematically in Figure 5. How bone remodeling units (tunneling osteoclast followed by osteoblasts) target damaged areas of bone is not understood. Osteocytes, the resident cells buried within the mineralized matrix of bone, appear to play a critical role in this process. Indeed, despite the widely held concept that bone remodeling functions in the repair of microdamage, empirical data demonstrating this basic physiological mechanism are scant, owing to the difficulty and complexity of performing such studies.

Burr, Martin, Schaffler, and Radin,[15] and Mori and Burr[77] showed experimentally that bone resorption spaces are associated with remodeling of linear microcracks in experimentally loaded canine compact bone. Recently, Bentolila et al.[5] reported an

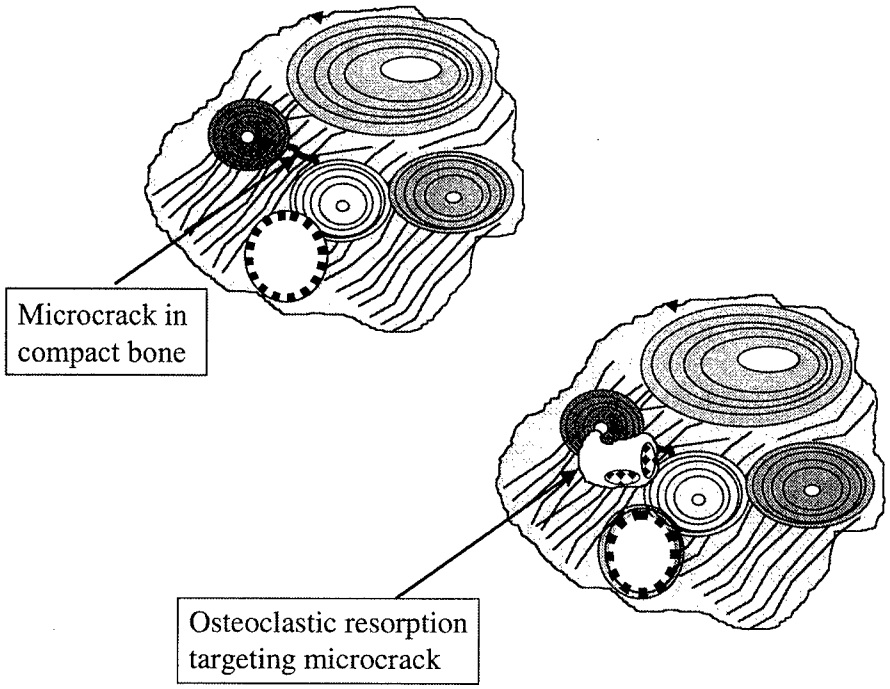


Figure 5 Schematic diagram showing microdamage in compact bone and targeted removal of the damage by osteoclastic resorption.

in vivo fatigue model based on end-load ulnar bending in adult rats, in which bone fatigue levels can be monitored as changes in whole bone stiffness. After fatigue loading, bone remodeling was activated and was observed in association with both linear microcracks and areas of diffuse matrix damage. Remodeling was effective in removing both damage types from the bone. Recent studies by Mashiba and co-workers[70] have taken a different approach to examining the relationship between microdamage and remodeling in normal bone physiology. They found that inhibiting bone remodeling in normally active dogs, using two types of bisphosphonate,[2,3] leads to a significant increase in bone microdamage content in the axial skeleton (ribs and vertebral bodies) as well as in long bones (femurs). These experiments show very convincingly that without an active remodeling-repair system, microdamage will accumulate in skeletal tissues as a result of normal, mechanically nominal levels of mechanical usage.

The cellular mechanisms by which groups of osteoclasts target regions of bone for resorption are unknown. However, it is reasonable to presume that osteocytes, the only cells embedded in the bone matrix, would be involved. Osteocytes and their elongated cell processes (in their lacunae and canaliculi, respectively) are widely and extensively distributed throughout the bone matrix. These cells are attached to their surrounding bone matrix with numerous attachment molecules, and to their neighboring cells through electrical connections known as gap junctions.[31,32] Osteocytes are highly responsive to mechanical loading.[63,103] Matrix disruption from

microdamage can be expected to directly injure osteocytes, disrupt their attachments to bone matrix, interrupt their communication through canalicular cellular and fluid flow processes, or alter their metabolic exchange. When osteocytes are lost from bone, complete fatigue fracture occurs. Examples include radiation-induced death of osteocytes,[72] allograft bone[6] and avascular necrosis.[60] Dunstan et al.[33] showed that the absence of osteocytes is associated with hip fracture. Osteocytes are focally lost in areas of microcrack accumulation in aging human bone.[86]

The involvement of osteocytes in bone fatigue and remodeling was recently demonstrated by Verborgt et al.[108] using the rat *in vivo* fatigue model. They found that with fatigue *in vivo*, osteocytes surrounding microcracks are injured and undergo an ordered cell disintegration process following a genetically regulated program, i.e., apoptosis. Regulated cell death is the ubiquitous biological process by which cells break down at the end of their functional life,[10,61,106] with the resulting cell breakdown products targeted by phagocytic cells. Osteoclasts belong to the phagocytic cell lineage. Osteocyte apoptosis has been observed in other metabolic situations associated with bone resorption.[9,79,106] Thus, osteocytes, and in particular the events surrounding their death, appear to provide a key part of the signaling process by which osteoclasts target microdamaged bone for removal and focal repair.

Left undetected and unrepaired, the accumulation of microdamage in bone leads to compromised mechanical properties and bone fragility. Damaged bone has significantly reduced mechanical properties in terms of strength and stiffness, and especially fracture toughness. Even small amounts of ultrastructurally based microdamage associated with early fatigue will compromise the functional-mechanical properties of bone. Fatigue damage has both mechanical and biological consequences. Stress fractures are the obvious application of the damage and repair concept in bone. However, bone microdamage, repair, and fragility are also implicated in bone aging, bone implant failure, and fractures associated with long-term usage of drugs that suppress bone remodeling physiology.[19,37,54,101] Suppressing remodeling may allow damage accumulation that will have deleterious mechanical consequences.

HOW DOES STRESS FRACTURE OCCUR?

There are two hypotheses regarding the causes of stress fractures. One hypothesis holds that stress fractures are the result of the accumulation and growth of microcracks within the bone. In this view, stress fractures are considered a purely mechanical damage occurrence, i.e., fatigue failure of the skeleton. However, fatigue to fracture as the primary mechanical causation for stress fractures is not supported by the experimental data (reviewed above). Alternatively, stress fracture has also been variously described as being primarily a biological process in which bone remodeling processes and periosteal reaction constitute the key features. However, there is little direct data on the pathophysiology of stress fractures. Attempts to understand the stress fracture process from human clinical studies have met with only limited success because of the inability to study bone tissue mechanisms directly. Mechanistic studies have not been performed in animals because of lack of a suitable experimental system until recently.

Of the few studies of human stress fracture tissues, those of Johnson and co–workers,[58] from the Armed Forces Institute of Pathology, stand out as the most critical in gaining insight into the physiology of stress fracture processes (also see Morris and Blickenstaff[78] for detailed discussion of Johnson's work). They obtained biopsies of stress fracture lesions from military recruits. Johnson observed woven bone reactions in numerous samples. Perhaps most significantly, however, focally increased intracortical remodeling was observed at stress fracture sites even in the absence of any woven bone response. Johnson's studies were based on histopathological biopsies of the lesions, taken at single time points, and therefore did not systematically examine the underlying development or physiology of the stress fracture lesions. Nevertheless, these data indicate that increased intracortical remodeling is one of the earliest and most prominent features in human stress fracture.

The association of remodeling and damage is supported by the stress fracture biopsy study presented by Mori in Chapter 10. Photomicrographs of the biopsy show accumulation of both diffuse damage and multiple linear microcracks in the region where the stress fracture occurred. Moreover, the bone surrounding the damaged regions is highly porous because of the presence of numerous active resorption cavities which are actively removing the damaged bone. These observations show that extensive microdamage is associated with the stress fracture and that bone mounts a repair reaction that will ultimately remove this damage.

Milgrom and co-workers in Israel[74,75] examined the time course of development of stress fractures among military recruits using serial ^{99m}Tc bone scans. They found that scintigraphic activity in bones destined for stress fracture increased significantly well before the existence of any increase in observable periosteal reaction. Early increased ^{99m}Tc uptake provides intriguing, albeit indirect evidence that increased bone turnover processes may be a significant early component in the development of stress fractures.

Recently, Stover and colleagues[104] reported histopathological data from racehorses with stress fracture that suggests that increased remodeling precedes the occurrence of microdamage in stress fracture. They obtained paired long bones from horses that had suffered complete (catastrophic) stress fractures of one limb. Cortical bones adjacent to the fracture sites showed elevated intracortical porosity. Most remarkable, however, is that comparable increases of intracortical porosity were also present at the same locations of the contralateral non-fractured long bones. Based on these findings, the authors suggested that increased intracortical porosity might be a necessary prerequisite to the development of stress fracture. As these were single time point studies, questions about the exact role of this increased bone turnover in the pathogenesis of stress fracture were not addressed.

Li et al.[66] reported experimental serial histological observations on the development of stress fractures in an animal model (Chapter 14). They produced stress fracture in rabbits by a chronic repetitive activity model. Animals were forced to jump and run in their cages for several hours per day for two months. Li et al. found that initial intracortical remodeling of the tibial diaphysis was the earliest observable change in the stress fracture sequence, with increased vascularity and osteoclastic resorption evident within the first week of repetitive loading. Periosteal reaction was not evident until the onset of intracortical resorption.

In our laboratory, we have developed an experimental animal model for stress fractures in rabbits, using repetitive impulsive loading of hindlimbs (Chapter 14).[12,16,102] Microfractures of trabecular bone and remodeling of the subchondral bone are a well established consequence of the repetitive impulsive loading model.[36,87,88] Adapted for use in diaphyseal bone, this model reproduces the scintigraphic and radiographic changes typically observed with stress fractures, including progressive increase in ^{99m}Tc uptake in bone, periosteal callus formation, and presence of microscopic cracks within the bone.[12,102] In this model, hindlimbs of skeletally mature rabbits were loaded to produce tibial diaphyseal stress fractures. Briefly, right hindlimbs were subjected to repetitive impulsive loading, using a cam-driven loading device. Loading is at 1.5 time body weight for a 50 millisecond cycle duration at 1 Hz. Animals receive 2400 load cycles daily. This regime causes stress fracture in the distal tibial diaphysis after five to six weeks of loading.

In the first series of experiments using this model, Burr et al.[12] showed that stress fractures in rabbits result from repetitive cyclic loading at low stresses. The lesions, which occurred in the distal third of the tibial diaphysis, were characterized at the organ level by progressive increases in bone ^{99m}Tc activity, followed later and variably by a periosteal reaction (Figure 6). In subsequent studies,[16] we measured tibial diaphyseal strains at the stress fracture site in the range of 500 to 1000 microstrain, which is within the normal physiological strain range (see above discussion). Strain rates, though increased somewhat over normal, were also within the range reported for normal locomotor activities.[93]

Recently, Schaffler and Boyd[102] examined bone tissue-level responses in the development of stress fracture in the rabbit stress fracture model. They showed that increases in intracortical porosity precede the accumulation of bone microdamage in experimentally induced stress fracture in this model. Intracortical remodeling at the stress fracture site was markedly increased by three weeks of loading, with the number of resorbing sites increased almost sixfold over control levels (Figure 7a). Intracortical remodeling activity was further increased by six weeks of loading, with resorption number increased more than tenfold over control levels). Resorption

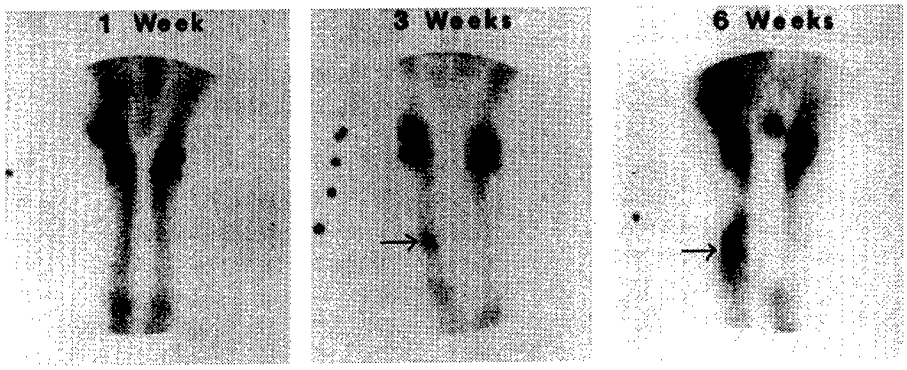


Figure 6 ^{99m}Tc bone scans of rabbit tibiae during development of experimental stress fracture. Arrows indicate increased isotope uptake in distal diaphyses of loaded limbs after 3 and 6 weeks of loading. Lesion severity progresses from 3 to 6 weeks.

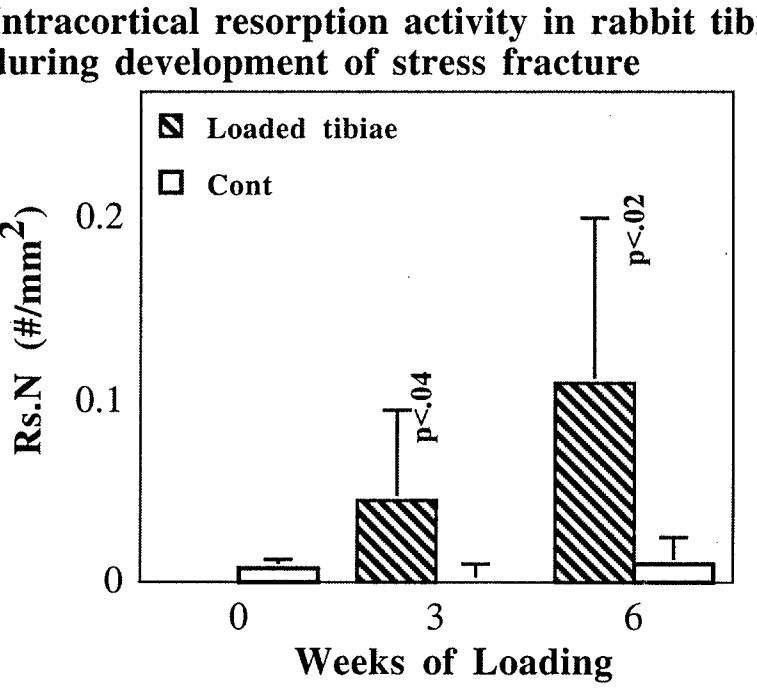


Figure 7a Intracortical resorption activity, as measured from resorption space number, at stress fracture site in rabbit distal tibial diaphyses after 3 and 6 weeks of repetitive loading. Significance values are shown relative to internal, nonloaded control limbs.

occurred primarily in the anterior and posterior tibial cortices, corresponding to the location of stress fracture and highest strain rate in this model. Bone microdamage was not observed in control bones or experimentally after three weeks of loading. By six weeks of loading, there was a significant increase in the number of microcracks observed in diaphyses Figure 7b. Typically, these were small cracks (mean length = 24 ± 7 μm). In addition, microcracks were observed only in those areas of the cortex that were undergoing intracortical remodeling (Figure 7c). Acute fatigue loading experiments, in which the equivalent of six weeks of loading was performed in one day, showed little microdamage induced by the loading alone (Figure 7b), confirming that rapid microdamage accumulation occurred only in the presence of increased bone remodeling.

The stimulus for activation of new remodeling sites in these experiments is not clear, as the experimental stress fracture site experiences a complicated series of changes relative to baseline in normal rabbit tibiae. These changes include altered strain distribution, increased loading rate with concomitant high frequency signal, and small amounts of microdamage, all of which have been shown to activate intracortical remodeling. Otter and co-workers[82] recently put forth the intriguing hypothesis that inadequate bone perfusion and reperfusion type injury in bone under chronic loading also might be a stimulus to activate bone remodeling in stress fracture. Thus, several lines of clinical, histopathological, and experimental data

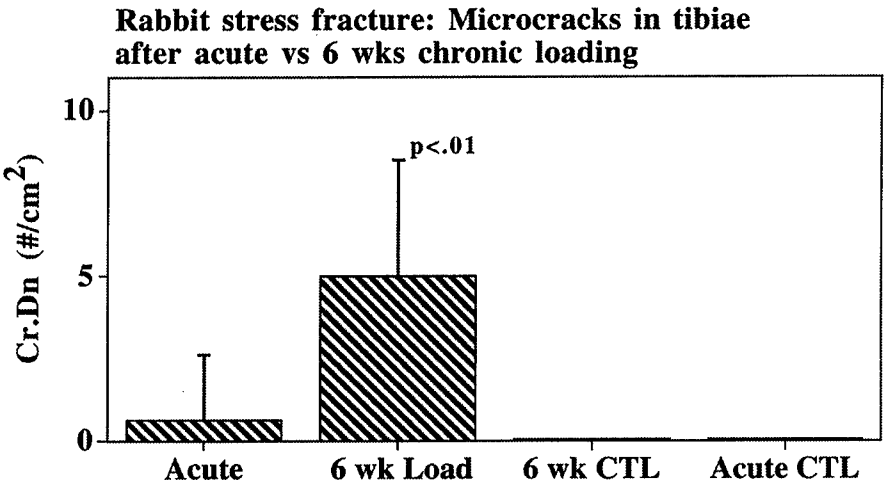


Figure 7b Microcrack content at stress fracture site in rabbit distal tibial diaphyses after 6 weeks of daily (chronic) loading versus acute loading (continuous loading for 20 hours to produce equivalent number of cycles to 6 weeks of daily loading). Acute loading, which occurs without increases in intracortical remodeling, results in a slight increase in bone microdamage content. Chronic loading, which occurs in the presence of significant increases in bone remodeling, causes a dramatic increase in microdamage.

show that increased bone remodeling occurs early in the stress fracture process. Activation of local remodeling activity results in focally increased bone porosity. Accordingly, increased intracortical porosity may be necessary for the later rapid accumulation of bone microdamage and development of stress fracture.

HOW CAN INCREASED REMODELING DRIVE MICRODAMAGE ACCUMULATION IN BONE?

A number of studies demonstrate that increased intracortical remodeling results from increased cyclic loading,[7,14,50,77] with direct mechanical effects (strain distribution, strain rate, frequency), matrix microdamage, and local cytokines among the possible stimuli for activating turnover. While the specific stimulus for activation of increased intracortical remodeling remains unclear, these studies all support the idea that early remodeling occurs with increased mechanical usage. In 1990, Schaffler, Radin, and Burr proposed a hypothesis for how elevated intracortical remodeling might drive the stress fracture process. They argued that increases in intracortical porosity, resulting from activation of intracortical remodeling, will have a dramatic effect on decreasing the stiffness of cortical bone. Continued loading of this focally osteoporotic bone will increase local stresses and strains, accelerate bone microdamage accumulation, cause periosteal hypertrophy and, ultimately, result in stress fracture. In essence, stress fracture would result when mechanical loading is sustained on a region of high turnover bone, creating a positive feedback loop leading to fracture, as summarized in Figure 8 (see Chapter 12).

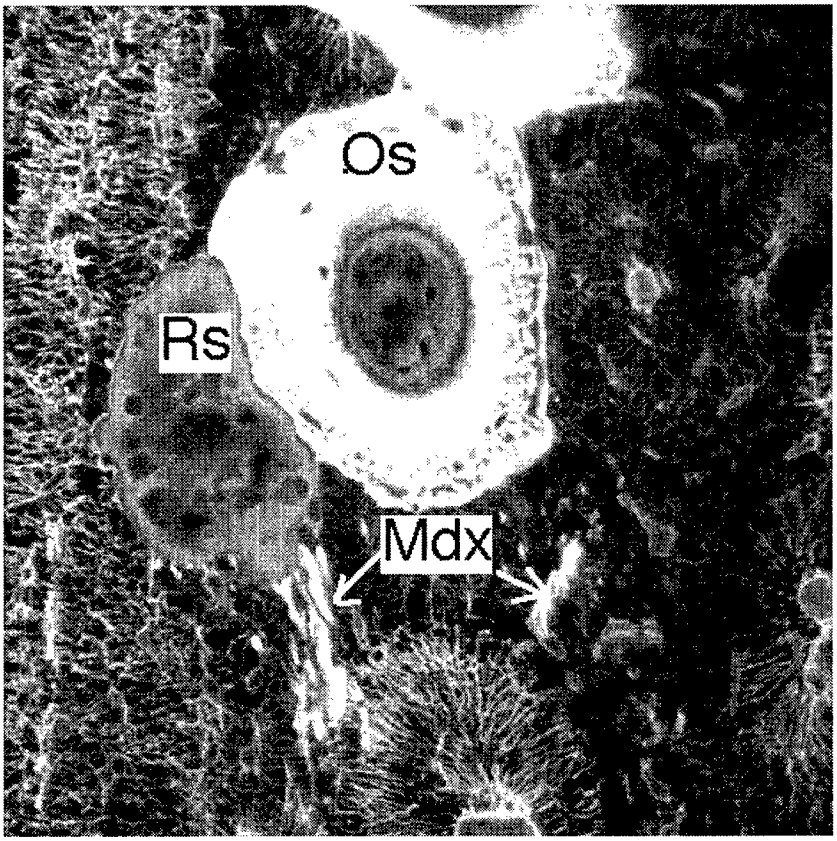


Figure 7c Confocal photomicrograph of rabbit tibial compact bone at 6 weeks of loading, showing intracortical resorption (Rs) and new osteon (Os) in association with bone microdamage (Mdx arrows) (Field width = 400 μm).

Intracortical remodeling begins by activation of new remodeling sites and recruitment of bone cells to the active surface. In the first phase of remodeling, osteoclasts resorb pre-existing bone, resulting in more and larger porosity within the cortex. In humans, the resorption phase is estimated to last for about six to seven weeks.[34,57] Thus, increased intracortical remodeling results in increased bone porosity, which lasts several months after onset. As a consequence of the increase in remodeling space, void (i.e., porosity) volume in bone expands at the expense of bone tissue volume (total tissue volume = bone volume + porosity). Numerous investigations have shown that stiffness of bone decreases with decreasing bone volume (or increasing porosity), following a power-law type relationship. In trabecular bone, stiffness is proportional to the cube of bone volume.[23] Compact bone stiffness is even more highly dependent on mass. Schaffler and Burr found that stiffness in compact bone decreases to the seventh power of decreasing bone volume, indicating that the stiffness of compact bone is profoundly sensitive to its porosity or bone volume.[95] Similar exponential relationships for compact bone stiffness and density/porosity

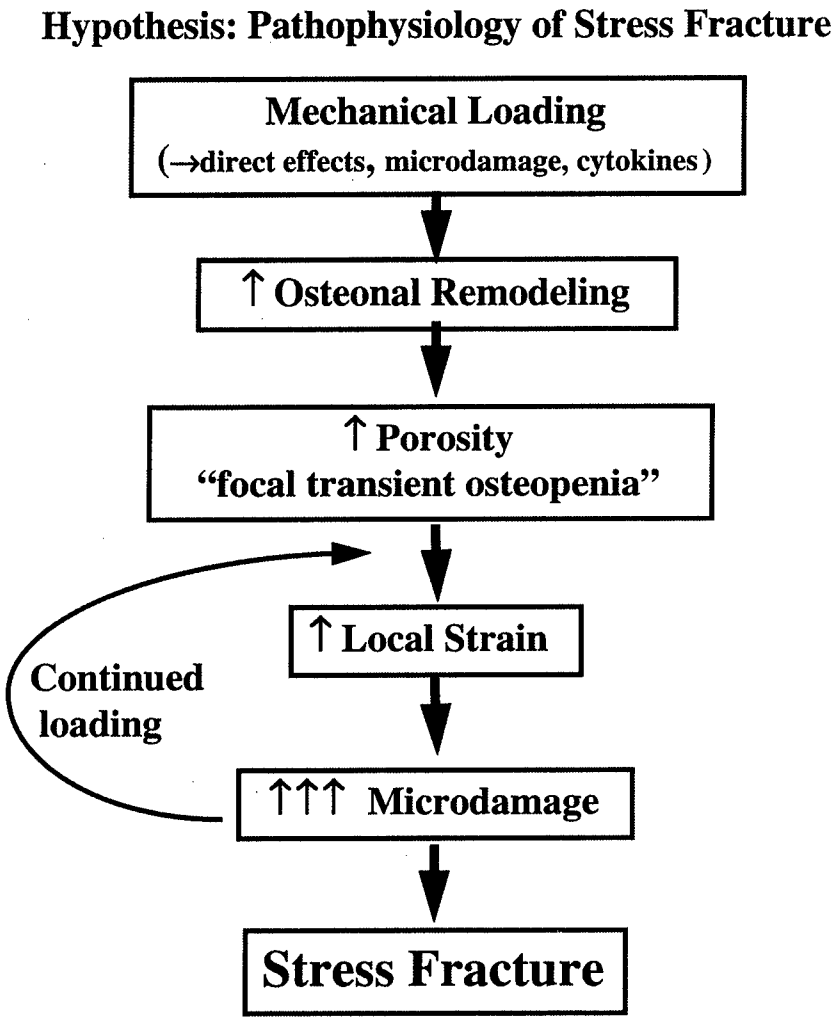


Figure 8 Schematic diagram summarizing the mechanism hypothesized for development of stress fracture, wherein increased bone remodeling and porosity (resulting from increased mechanical usage) are a prerequisite for the development of stress fractures. Increased local strains during continued loading would accelerate the accumulation of bone microdamage and the development of stress fracture in a positive feedback type manner.

were reported recently by Les et al.,[65] confirming that compact bone stiffness changes dramatically in response to small changes in intracortical porosity or bone volume.

The recent mathematical model for stress fracture development by Martin[68] is of particular interest in this regard (Chapter 12). Using a feedback model to examine the effects of increasing porosity on the mechanical properties of compact bone and development of stress fracture, Martin showed that there is a critical porosity — load interaction threshold. Once this point is reached, through increased bone porosity

and/or through increased local loading, Martin demonstrates that the system becomes unstable (i.e., positive feedback), and bone fails rapidly and catastrophically.

In summary, experimental data and several lines of clinical, histopathological data support the idea of a complex interplay between mechanical loading and bone remodeling in the etiology of stress fractures. While bone readily sustains fatigue microdamage during the course of repeated loading at the stresses or strains encountered in normal activities, it does not lead to fracture in the time course seen for the development of stress fracture. The model that best explains the development of stress fracture is that of a biologically (remodeling) driven damage accumulation system. In this model, stress fracture occurs as a positive feedback mechanism (Figure 8), wherein increased mechanical usage stimulates bone turnover, which results in focally increased bone remodeling space (porosity) and decreased bone mass. There is a wide range of factors (low level fatigue, altered mechanical loading, injury, cytokines, vascular) that can potentially activate local bone remodeling. All of these can occur in the development of stress fracture. With continued loading of this focally, transiently osteopenic bone, local stresses would be markedly elevated, leading to accelerated matrix damage and failure. Fracture is the result of continued repetitive loading superimposed on the decreased bone mass caused by more and larger resorption spaces

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